The Journal of Parasitology

Volume 5 JUNE, 1919 Number 4

FASCIOLOPSIS BUSKI

A PARASITE OF MAN AS SEEN IN SHAOHING, CHINA *

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THE DISEASE

Fasciolopsis infestation of man, for many years a medical curiosity, has within the last decade come to be recognized as a serious condition both for the individual and for the community concerned. The first flukes of this type to be described were discovered by Busk in the intestines of a Lascar sailor dying in England in 1843. The parasite has been found in a few other cases in Europe and America, but apparently the disease is endemic only in tropical and subtropical countries. As these comparatively little known regions become more deeply penetrated by the medical missionary and others, infestation of man by this species or related ones is found with increasing frequency, and is now known to occur in India, Assam, Siam, Natal, Borneo, Straits Settlements, Sumatra, Cochin-China, Tonkin, and along the coast of China (Canton, Hongkong, and as far north as the Yang-tse Valley, where in the Shaohing district it is particularly prevalent). In pigs *Fasciolopsis buski* has been reported to be very common in Hongkong and Tonkin. In man, at least in Shaohing, the disease appears to be more common in early life, 5 to 20 years, but it is not rare in persons up to middle life, and we have found it in one infant but a year old.

The prevalence of this disease in any community will in all probability depend on the dietetic habits of the people. Dobson reports that 1 per cent. of the stools of 1,000 coolies examined in India showed the eggs present; Mathis and Léger speak of the "extreme rarity" of the infection in man in Cochin-China. In Shaohing, China, it is extremely common. In the seventeen months from January, 1908, to May, 1909, the diagnosis of fasciolopsis infection was confirmed by microscopic examination of the feces in 5½ per cent. of all the dispensary patients, about 2 per cent. more presented very suggestive symptoms, and doubtless many others were harboring the parasite, though without symptoms. Shortly after the in-patient department of the hospital was

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opened nineteen out of twenty patients, or 95 per cent., showed ova in the feces, though none of the twenty was admitted for that condition, nor even suspected it. During the years 1914-16 out of 304 cases admitted to the Christian Hospital, and in which routine fecal examinations were made, 87, or 28 per cent., were found positive for the presence of fasciolopsis.

Three stages may be recognized clinically.

1. *The Period of Latency.*—This period is without notable symptoms and may occupy months or even years. A few flukes in the intestine seem to cause no inconvenience, and how severe the infection needs to be before giving rise to symptoms it would be impossible to say; but in the Christian Hospital the number of flukes recovered from the stools of an individual has varied from a few tens to over three thousand (3,328), and while in general the severity of symptoms is proportional to the number of flukes present, there is also a marked difference in the degree of resistance that an individual may possess, some from whom but a few hundred were recovered being clinically in worse condition than those who had harbored as many thousands. It is probable that asthenia in varying degree, and a mild anemia may appear toward the termination of this stage.

2. *The Period of Diarrhea.* — During the second stage diarrhea appears, and is the condition for which most often relief is sought. There is generally a history of five or six stools a day extending over a period of months, and often with intermissions of days or weeks during which the bowels act normally. The stool itself is usually light yellow in color, without any evidence of blood even under the microscope in uncomplicated cases. It contains a considerable amount of undigested matter, and has a peculiarly offensive odor. Anemia now becomes noticeable and may be extreme, and in Shaohing the combination of anemia with chronic diarrhea is practically pathognomonic of fasciolopsis infection. Other symptoms are inconstant. The appetite may be impaired or even increased, but is usually unaffected; occasionally there may be dull aching pain distributed throughout the abdomen or localized in the duodenal region; temperature, pulse, and respiration do not in uncomplicated cases differ from the normal. In infants and young children the abdomen is very protuberant, and may be the first evidence of disease noted by the parents.

3. *The Period of Edema.*—During the third and final stage anemia is always marked but the most prominent as well as most distressing symptom is edema. This usually affects first the abdominal cavity, then extends to the genitals (which may be very greatly distended), and to the lower extremities beginning in the feet and ankles but soon involving the whole limb, and finally appears in the upper extremities,

the face and the lungs, and with these advanced conditions insufficiency of the cardiac valves may supervene. To relieve the dyspnea due to the ascites it may be necessary to resort frequently to paracentesis abdominis, sometimes as often as every five or six days for a considerable period. The urine is normal except for undue concentration, and even under diuretics may be reduced to but two or three ounces in twenty-four hours. The skin has a yellowish tinge, is harsh and dry, the tongue is glazed, the temperature has a tendency to fall one or two degrees below normal, the patient become extremely weak and death when it occurs is apparently due to exhaustion.

In the treatment of this condition turpentine, oil of eucalyptus with chloroform, thymol and beta-naphthol have all been used with success, the treatment being in fact the same as for hookworm. Personally, I favor beta-naphthol, and believe it better practice, on account of the marked depression which occasionally comes on even when least expected, whatever drug is employed, to give small doses repeated as often as necessary, rather than to attempt to expel all the parasites in one or two doses. Oil of chenopodium has not yet been tried, because it was unavailable when called for. Restricted diet and saline purgatives both before and after are of course indicated. Dead flukes will usually begin to be passed within twelve hours after taking the anthelmintic, and will continue to come for two or three days. Tonics are rarely needed, as it is astonishing how rapidly the anemia disappears once the intestines are cleared.

Prophylaxis, naturally, will consist in the avoidance of uncooked food. The life history of this fluke is still to be worked out, but the fact that fresh water snails enter largely into the diet of the people of this region, and that before being eaten they are subjected to only slight scalding, is suggestive that these molluscs may be the secondary host. Shrimps have also been considered, but they are in general quite thoroughly cooked.

THE PARASITE

The parasite in question exhibits such wide variations in morphology that four species have been described, and recently Brown has suggested a reclassification into two groups differentiated chiefly by the presence or absence of cuticular spines. The literature on the subject is somewhat contradictory and confusing, and until recently the material has been so scanty and appeared at such great intervals of space and time, that a restudy of the subject in the light of considerable clinical experience and with a larger amount of laboratory material seemed likely to yield important results. The following report, which is preliminary to further studies on the life history of the parasite and its effects on man from the pathological standpoint, is based on a

practice extending over several years in a heavily infested district (Shaohing, China), supplemented by a laboratory study of 433 specimens. Twenty-one flukes have been cleared, the ventral sucker with cirrus sac and metraterm attached have been dissected out from three, ova have been removed for measurement from the lower uterus of nine, and seventeen series of microscopic sections have been made of individuals conforming to the descriptions of the three types which have been described as *F. buski, F. rathouisi* and *F. goddardi,* including five serial sections in different planes.

In selecting specimens as representatives of each type, in general, those were chosen which in size fell within the published measurements; viz. a length of 25 mm. or more for *F. buski;* 21-24 mm. for *F. goddardi,* and 15-20 mm. for *F. rathouisi.* But a few exceptions were made where other physical features clearly indicated a different classification. This fact will explain the apparent inconsistencies in the grouping, and illustrate at the same time the extreme difficulty encountered in making any distinctions on account of the many borderline cases.

In summarizing the results of this study, it will be convenient to consider first the variations upon which differentiation into species has been based; and to conclude with a somewhat detailed description of the morphology as a whole, especially of those features about which there has hitherto been doubt.

The variations which have figured in the differentiation into separate species may be grouped as follows:

1. *Color and Consistency.*—Specimens preserved in alcohol vary in color from brown to grayish-white, and frequently the vitellaria are clearly outlined by a bluish black pigmentation along the lateral and posterior margins (Pl. XII, A). Some are firm in consistency, while others are flabby and soft. These variations are found in individuals of all the types of this genus which I have examined, and are undoubtedly due to postmortem changes. The fluke when alive or freshly killed is of a deep pink color, not unlike that of boiled ham, and the great majority of all flukes recovered from the stools are of this color and are firm in consistency. Occasionally flukes are passed which are pearly white with dark borders, and are flabby in appearance, and to the touch. Under the microscope such specimens are seen to have lost their cuticula (Pl. XIV, *A*), and the cells of the yolk glands have fallen away from the basement membrane of the acini and are collected in a more or less disintegrated mass in the lumen, the nuclei being deeply pigmented. Recalling the fact that flukes are often two or three days in the intestine after the anthelmintic has been taken, it is easy to understand that partial digestion of the surface has occurred, quite sufficient to account for the phenomena observed. Similar but

less marked changes occur in specimens allowed to remain for a considerable time in water before being placed in preservative.

2. *Size and Shape.*—Variations in size and shape are so extreme (Pl. XII, *A*) as to warrant a belief in the existence of more than one species until it is found that gradations from one to another type are so gradual as to make lines of demarcation quite impossible.

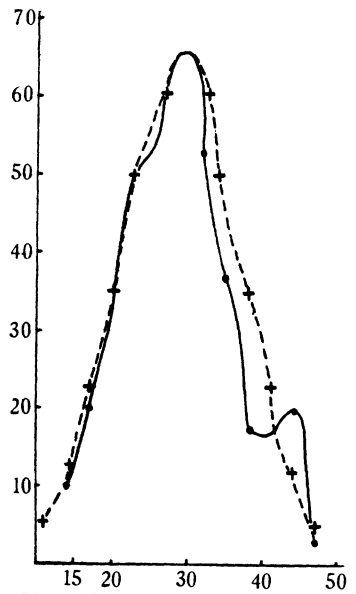

Fig. 1. Length frequency curve constructed from measurements of 378 individual flukes.

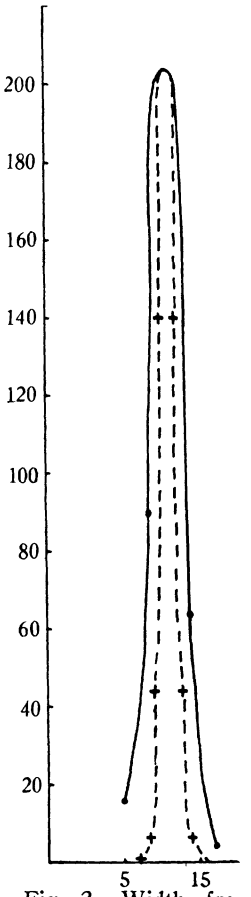

Fig. 2. Width frequency curve.

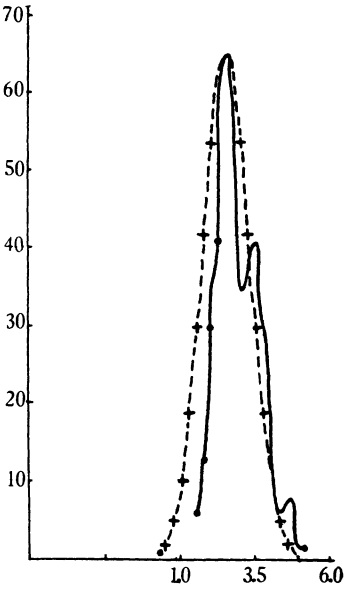

Fig. 3. Proportion frequency curve, i. e., ratio of length to width.

In this investigation measurements were made of 378 flukes ranging from 13 to 48 mm. in length and from 4 to 17 mm. in width. Progression in length and width measured in millimeters, and in the ratios between them, measured in $\frac{5}{100}$ of the width was found to be perfectly even. Frequency curves were then plotted and are shown in Graphs 1, 2 and 3. A certain amount of error is possible here, due to the fact that the flukes were not all in the same state of muscular contraction when fixed, but all the curves, for length, for width, and

for proportion, are so distinctly unimodal, and conform so closely to the corresponding theoretical curves for variations* as to leave little doubt that we are dealing with a single species.

The thickness measured at the middle varies in this series from 0.8 to 3.0 mm., while over the acetabulum, the thickest part, the maximum found was 3.5 mm., and the variation is somewhat less. No relation can be found between the thickness and the various suggested species. That described as *F. rathouisi* is characteristically "short and stocky"—one such measured 20x12x2.8, was brown in color, firm in texture, and in a state of opisthotonos. But another measures 19x13x2.0, is glistening white with dark borders, and lies flat and relaxed. On the other hand, a fluke (*e* in Pl. I, *A*) typical of *F. buski,* measured 43x12x1.0, but others measuring 40x9x2.3 or 32x10x2.3 or 33x10x3.0 also occur.

In part, these differences may be ascribed to natural variation, but some are certainly due to the state of muscular contraction at the time of fixation. This is apparent not only from the numerous and deep transverse rugae which are seen frequently in preserved specimens (cf. *i* and *j* in Pl. XII, *A*), but also from observations on flukes in the fresh state. In one instance 22 flukes were brought in immediately after evacuation, all of which appeared to be long and narrow (about 35x8 mm.) and many of which showed an inrolling of the edges of the cephalic portion about the long axis so as to form almost a complete sheath. I was interrupted in taking their measurements, and after remaining in water four hours all were found to have assumed the ordinary appearance, varying in length from 18 to 35 mm., in width from 13 to 20, the average being 28.6x16.4, and extremes being 35x20, 18x16 and 28x13. On another occasion a fluke measuring 28x19 on evacuation, was found after standing two days in water to measure 41x17.

3. *Head Cone.*—In none of the Shaohing flukes is there a distinct "head cone" or shoulder such as occurs in *Fasciola hepatica,* for example; but in certain specimens (cf. *i, g, f* in Pl. XII, *A*) there is a narrowing at the level of the genital pore, and when viewed from the side a posterior bulging. This is due to the contraction of the dermo-muscular tube and parenchymal muscles closely about the solid muscular bodies of the region (oral sucker, pharynx and acetabulum), and is not distinctive of any of the three types.

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* The normal curve in all these figures is plotted according to the commonly used formula

$$y = \frac{n}{\sigma\sqrt{2\pi}} \cdot e^{\frac{1}{\frac{x^2}{2\sigma^2}}}$$

4. *The Cuticula.*—In the early description of *F. buski* by Cobbold the integument is said to be "smooth and unarmed," and Odhner, Ward, Looss, Braun and Rodenwaldt either state that spines are absent or adopt the view generally held that they are absent. Heanley and Jefferys state positively that they are present "though very difficult to find in some mounted specimens" (Heanley), and Leiper says they are present in the species *F. rathouisi* and *goddardi,* and explains their occasional absence from certain specimens by the "deciduous character" of the cuticula, claiming to have seen in a section of Rodenwaldt's specimen the regularly recurring pits in the cuticula from which the spines have dropped out. More recently Brown has found an apparent coincidence between the presence or absence of spines and the marked differences in gross appearance already discussed, and suggests a reclassification of the several varieties now named into two groups on the basis of this characteristic.

Of the present series of 433 flukes, half (including all the variant types illustrated in Pl. XII, *A,* and among them typical examples of the two groups suggested by Brown), were examined by strong reflected and transmitted light, and in all of these spines were found though in many cases only with difficulty and in very small numbers, due to the deciduous character of the cuticula to which Leiper has already called attention.

Of the flukes photographed in Plate XII, *A, e, f, j* and *k* have since been imbedded and sectioned. In *j* and *k* the cuticle is well preserved and the spines numerous; in *e* and *f,* on the contrary, the cuticle, except in a very few scattered spots, is entirely gone. A comparison of Plate XII, *B,* Plate XIII and Plate XVII, *A,* shows how readily even in fairly well preserved specimens the cuticula strips off from the basement membrane carrying the spines with it. These facts furnish a satisfactory explanation of the occasional failures hitherto to demonstrate the presence of cuticular spines, and warrant the conclusion that they are charactersitic of the entire genus.

5. *Form and Size of Various Viscera.*—The cirrus sac is convoluted in all specimens, varying from two or three close turns like the strands of a rope to larger spirals, and its course may be straight or sharply bent upon itself; but neither these variations nor the relative length of the sac posterior to the acetabulum are characteristic of any group, being found in each. The diverticulum of the seminal vesicle, the so-called "cecal appendage" was present in all specimens examined.

The shellgland is an elastic body, and though normally ovoid in shape, is capable of being markedly affected by the surrounding muscles; appearing in one highly contracted specimen (fluke *m*) as an antero-posteriorly flattened disk extending the entire dorsoventral diameter of the body.

TABLE 1.—VISCERAL MEASUREMENTS IN MICRONS. LETTERED INDIVIDUALS REPRESENT SECTIONS; THE OTHERS CLEARED FLUKES

	Flukes: Cleared and Sectioned	Oral Sucker		Pharynx	Genital Pore	Ventral Sucker			Shell Gland			Cirrus Sac*		Testes	Vitelline Acini	Remarks
		Whole Organ	Orifice			Whole Organ	Orifice	Distance from Head	Size	Distance from V. S.	Distance from Head	In Microns	Per Cent.			
F. buski	45×13×1.3	876×876	350	759×992	.....	3,212×2,569	876×1,518	1,460	1,401×1,576	6,424	15 mm.	3,796	59	292–759	58–175	
	33×8	350×467	175	467×700	116×292	2,511×2,102	1,051×1,216	1,752	992×1,168	7,884	12 mm.	4,672	59	? –642	58–146	
	29×11	408×584	280	408×759	234×408	2,920× ?	876×1,460	1,284	1,109×1,226	7,416	12 mm.	4,204	54	116–700	58–116	Cirrus sac very angular course
	27×9	.....	...	.....	175×408	2,336× ?	467× 584	1,284	934×1,168	6,716	12 mm.	3,212	47	116–525	58–175	
	29×13	700×817	408	584×700	.....	2,628×2,336	.....	1,576	.....	.....	11 mm.	4,672	76(?)	116–525	58–120	
	39×11	700×876	350	642×700	292×584	3,212×1,752	1,284×1,343	1,868	1,343×1,460	9,928	16 mm.	4,555	45	116–700	58–175	
	31×14×2.3	700×700	467	584×759	? ×408	2,336×2,044	876×1,168	1,868	642× 876	7,884	13 mm.	4,380	55	175–584	58–116	
	35×9 A	430×619	378	344×498	*292×516	2,752×2,150	.....	.....	1,204×1,720	7,740	.....	5,160	67			
	43×12 E	602×619	326	550×584	*326×430	2,924×2,201	.....	.....	1,548×1,548	9,718	.....	5,160	53	.....	.....	Slightly irregular rounded
	16×? P	430×240	...	395×481	* 86×172	1,874×1,100	.....	.....	860× 820	4,644	.....	2,408	52	.....	.....	Sagittal section
	40×9×2 N F	430×550	292	498×567	51×172	2,854×1,823	.....	.....	946×1,376	8,944	.....	4,816	54			
F. rathi.	15×10	58×292	...	.....	*408×467	2,336×1,752	584×1,460	.....	700× 876	.....	7 mm.	.....	..	175–408	.....	Cirrus protruding
	21×11	292×350	...	467×700	116×350	.....	292×1,284	.....	759×1,168	4,263	9 mm.	.....	..	.....	58–175	
	19×11	525×759	292	642×817	175×350	? ×2,044	876×1,401	.....	.....	.....	.....	.....	..	.....	58–116	
	16×7 L	344×361	120	309×447	34×120	1,892×1,513	.....	.....	516× 688	4,558	.....	2,494	56			
	20×12 M	344×258	...	464×430	.....	2,476×1,462	.....	.....	946×1,720	4,988	.....	4,128	83	.....	.....	Sagittal section, greatly contracted
F. goddardi	22×10	467×525	292	.....	175×408	2,452×2,044	525×1,168	1,168	1,168×1,284	4,672	9.5 mm.	2,628	56	? –584	58–175	
	23×10	580×580	292	.....	233×467	2,511×2,044	642×1,401	1,051	1,460×1,460	5,256	11 mm.	3,679	69	? –408		
	22×11	584×700	292	.....	.....	2,044×1,635	817×1,109	.....	876× 876	.....	10 mm.	.....	..	116–350	58–116	
	22×9	.....	...	.....	.....	2,336×1,752	580× 700	1,284	934×1,343	6,716	10 mm.	3,796	55	116–350		
	20×6 B	.....	...	.....	.....	2,201×1,444	.....	.....	.....	6,192	.....	2,408	40			
	23×9 C	412×412	240	619×636	*172×395	2,580×1,806	.....	.....	946×1,290	7,098	.....	3,658	51	.....	.....	Cirrus protruding
	23×? H_1	447×412	86	395×567	*103× ?	2,322×1,462	.....	.....	946×1,118	5,160	.....	3,440	66	.....	.....	Sagittal section, cirrus protruding
	23×8 H_2	533×602	344	.....	*154×516	2,081×1,840	.....	.....	1,118×1,118	4,472	.....	3,010	68	.....	.....	Slightly heart shaped
	25×8 J	688×516	103	584×705	64×206	2,958×1,720	.....	.....	946×1,032	4,128	.....	1,892	47	.....	.....	Sagittal section, contracted
	23×10 K	.....	...	464×447	.....	2,150×1,462	.....	.....	1,118×1,238	7,740	.....	3,612	46			
	20×6 O	378×430	223	516×567	*154×240	2,304×1,892	.....	.....	946×1,204	4,816	.....	2,408	50			

* First column is length of sac posterior to acetabulum; second column is ratio of this length to entire distance between acetabulum and shell gland.

Measurements of the various organs are tabulated in Table 1, from which it appears that the variations which occur are not characteristic of the groups, but rather bear a general relation to the size and development of the individual.

6. *The Ova.*—Observation of the ova in hundreds of samples of fresh feces leaves an impression on one of their essential unity, an

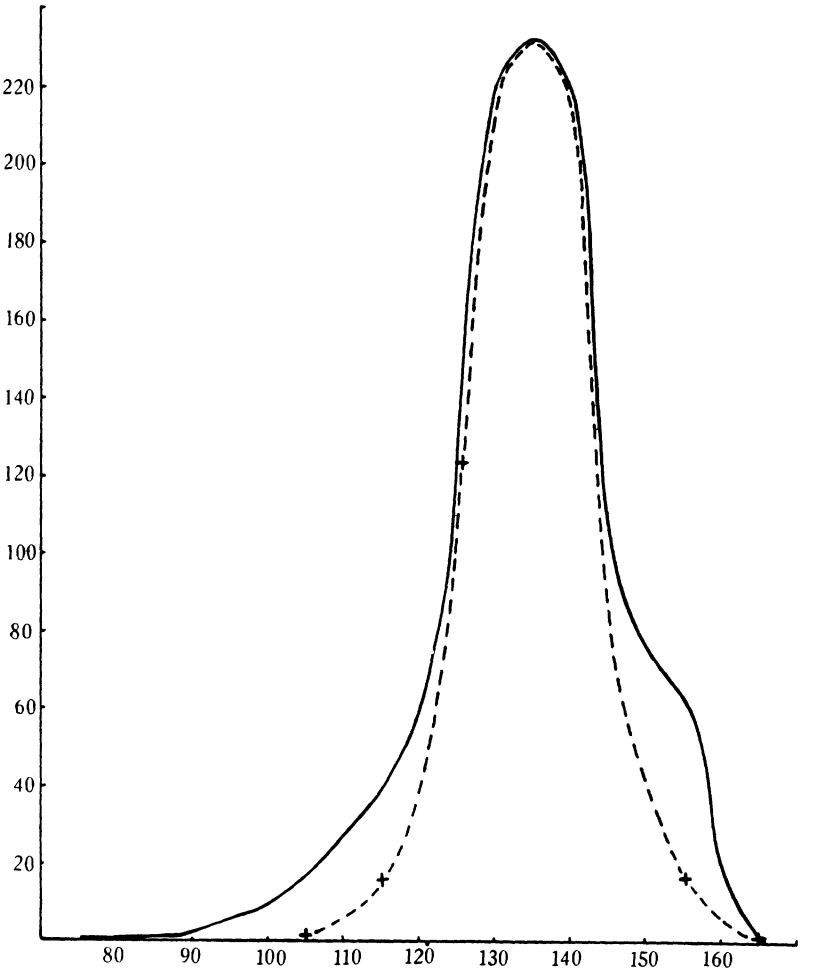


Fig. 4. Curve representing length of ova taken from entire series.

impression confirmed by the present series of measurements. In all, 576 eggs were measured, of which 150 were from two separate samples of fresh feces, and the rest from the lower uteri of nine preserved flukes, including representatives of each variety. The entire series is found to be grouped, in length, about the single mode 130-140μ with variations conforming fairly well to the normal curve (Graph 4). The same mode, it will be noted, obtains for each of the groups as well,

TABLE 2.—TABLE OF MEASUREMENTS OF OVA

Source	No.	Length in Microns										Width in Microns												Remarks
		71/ /80	81/ /90	91/ /100	101/ /110	111/ /120	121/ /130	131/ /140	141/ /150	151/ /160	161/ /170	51/ /55	56/ /60	61/ /65	66/ /70	71/ /75	76/ /80	81/ /85	86/ /90	91/ /95	96/ /100	101/ /105	106/ /110	
F. buski																								
1. 40×11	79	...	...	..	...	...	21	58	...	...	...	...	...	...	...	...	...	4	9	42	12	6	6	
2. 27× 9	28	...	...	..	...	2	16	10	...	...	...	...	...	...	2	7	16	0	0	2	0	0	1	Distortion, swelling, asymmetry present
Group b	...	...	...	..	...	2	37	68	...	...	...	...	...	...	2	7	16	4	9	44	12	6	7	
F. rathouisi																								
3. 17× 5	30	...	...	..	4	8	18	...	...	...	...	...	...	1	5	3	10	5	5	1	..	...	...	Some swelling
4. 13× 8	30	...	...	..	...	14	16	...	...	...	...	...	...	...	...	...	20	9	1					
5. 17× 9	30	...	...	..	...	3	12	15	...	...	...	...	...	1	4	6	16	0	2	1	..	...	...	Slight asymmetry
6. 22×13	100	...	...	..	...	...	1	51	38	10	...	...	...	...	...	...	50	41	9					
Group r	...	...	...	..	4	25	47	66	38	10	...	...	...	2	9	9	96	55	17	2				
F. goddardi																								
7. 25× 9	30	1	1	5	14	7	2	...	...	...	...	1	...	1	...	...	2	3	4	9	4	4	2	
8. 24×12	50	...	...	..	...	...	1	7	24	18	...	...	...	...	...	...	24	19	3	4				
9. 23×12	49	...	...	..	...	...	...	4	12	32	1	...	...	...	...	...	8	14	20	7	..	...	...	General swelling, frequent asymmetry
Group g	...	1	1	5	14	7	3	11	36	50	1	1	...	1	...	...	34	36	27	20	4	4	2	
Feces																								
1. Feb. 1909	50	...	...	1	...	1	5	28	13	2	...	...	...	...	4	...	11	31	...	2	..	2		
2. Sept. 1917	100	...	...	..	1	5	14	64	14	2	...	...	...	...	1	3	65	23	8					
Group f	...	...	...	1	1	6	19	92	27	4	...	...	...	...	5	3	76	54	8	2	.	2		
Total	576	1	1	6	19	40	106	237	101	64	1	1	...	3	16	19	222	149	61	68	16	12	9	
Series 2	497	...	. .	1	5	33	104	233	89	32	...	...	...	2	16	19	212	132	37	50	12	8	7	Nos. 7 & 9 omitted
Group 2																								
Nos. 1, 2, 3, 7	...	1	1	5	21	17	57	68	...	...	...	1	0	2	7	10	28	12	18	54	16	10	9	
Nos. 4, 5, 6, 8, 9	...	...	. .	.	. .	17	30	77	74	60	1	...	..	1	4	6	118	83	35	12				

except Group G, in which it is raised somewhat by the abnormality discussed below. If now on account of the great divergence shown by individuals in this group and for other reasons we throw out *F. goddardi* altogether, and make but two species as shown in Group 2 of the table (Table 2), here again both species—*buski* and *rathouisi*—

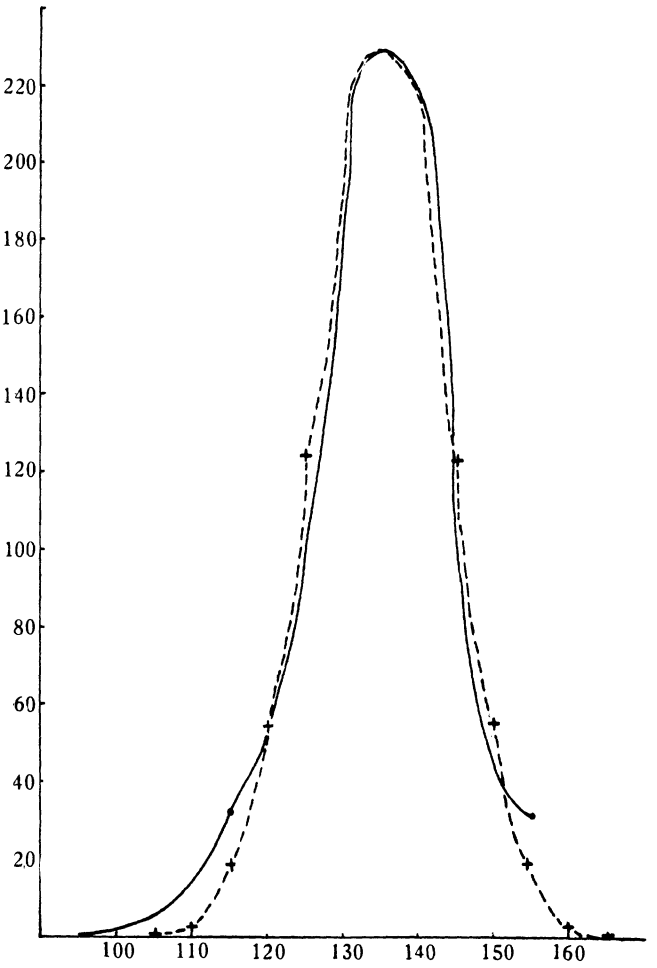


Fig. 5. Curve representing length of ova in amended series.

are found to have the same mode. It is also noteworthy that eggs deposited in feces—a normal condition—form a curve which agrees very closely both with its own normal and with the curve for the entire series, and has a much smaller standard deviation—spreads less —than do any of the curves for eggs from an individual. Graphs 4-7 graphically represent this fact, which is strikingly apparent under the microscope, viz., that ova taken from the body of the parasite present

far greater variations in contour and in size than are seen under normal conditions. In particular, such variations in contour were especially noticeable in eggs from fluke 9, and a large proportion of them were apparently swollen; i. e., a clear space appeared between the yolk contents and the shell, the former remaining normal in size and shape, while the shell measured from 23-27μ more in length, and about 11 more in width. To a less extent these conditions were found also

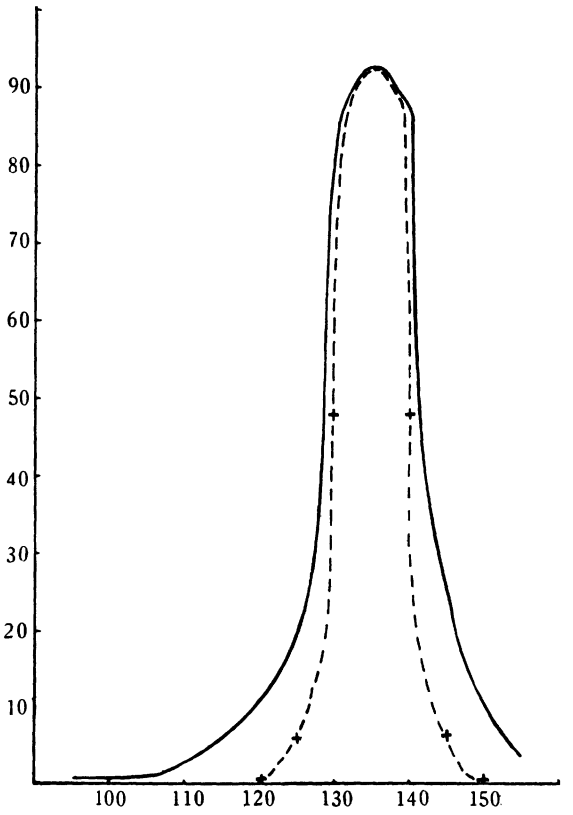


Fig. 6. Curve representing length of ova taken from feces.

in flukes 2, 3 and 5, and are doubtless abnormalities, as I have never seen them in feces specimens. On the other hand, eggs from fluke 7 were relatively so few (i. e., the total number obtained from the uterus were so few), and are grouped about a mode so much smaller than the average that they may fairly be considered immature, especially since in another fluke of about the same dimensions practically no eggs were found. If now on the supposition that they are abnormal, the eggs from these two flukes, 7 and 9, be excluded from the series, and the curve of the remainder be plotted as is done in Graph 5, the close correspondence between observed and theoretical curves furnishes

conclusive evidence of the correctness of the hypothesis. Further, that these variations are individual abnormalities rather than indications of specific differences seems clear from the fact that in the entire series they stand alone, that the flukes from which they were taken are in other respects indistinguishable from each other and from flukes yielding normal eggs, and that the eggs differ as described above from any that I have seen that were deposited in the normal manner. In the above discussion the short diameter of the egg has not been considered because it does not alter the problem. Those measurements, however, will all be found in the table (Table 2).

Writing in the Encyclopaedia Britannica, Peter Chalmers Mitchell defines a species as "an assemblage of organic forms which . . . if they differ among themselves differ less markedly than they do from

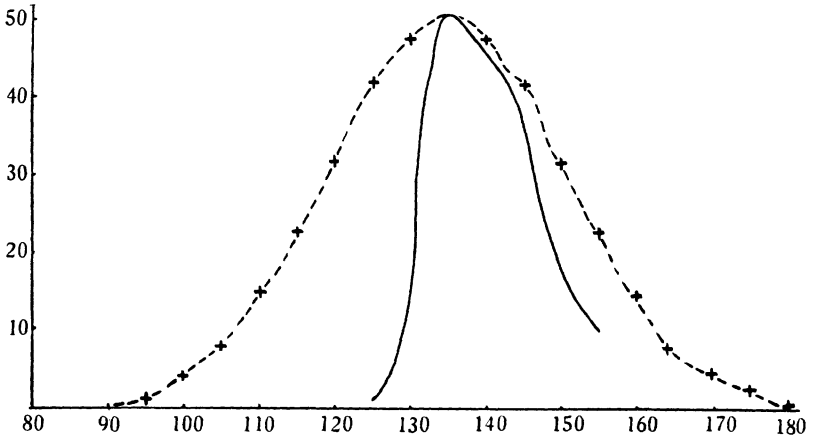


Fig. 7. Curve representing length of ova taken from fluke No. 6. For further details compare text.

those outside the species, or if differing markedly are linked by intermediate forms." With such a definition and in the light of the foregoing discussion it would seem that the three varieties represented in the Shaohing specimens should be regarded as belonging to but one species, *F. buski.* Poirier's original specimen on which the species *rathouisi* was based, came from a suburb of Shanghai, the commercial metropolis of the region, to which people from neighboring cities have flocked in large numbers. In recent years a few cases of fasciolopsis infection have been reported from Shanghai, and some of them at least are known to have been natives of Shaohing. It is therefore quite probable that Poirier's specimen came from Shaohing, which if true would tend to support the argument from morphology presented by Odhner and others to the effect that it was in reality but a contracted form of *F. buski.* Kwan's fluke has been conclusively shown by Leiper to be a mutilated specimen of the same species, and the

description of *F. fülleborni,* given by Rodenwaldt, agrees with that of *F. buski,* as modified by the present study in every particular except that it is said not to possess cuticular spines nor the characteristic diverticulum of the seminal vesicle. If Leiper's statement, already quoted, regarding spines in this case be accepted, there remains but a single point of difference outstanding; and in view of the limited amount of material (three specimens from a single case) on which the description was based, further study would seem to be required before the identity of this as a separate species can be accepted.

MORPHOLOGY OF THE PARASITE

Assuming then the existence of but a single species in the Shaohing flukes, the following account of its morphology may be given, which applies equally to all varieties, except that measurements of internal parts are to be understood as applying to individuals approximating the average in size.

General Appearance.—F. buski is a flat, elongated fluke, typical specimens measuring about 30x12x2 mm. Busk reported a maximum length of three inches (75 mm.?). In Shaohing the greatest measured length was 55 mm., and the greatest width 20. Immature forms 5 mm. or less in length have been recovered from feces, and in six uncontracted forms measuring from 9 to 21 mm., but a few poorly developed eggs were found in the longest, and in the others none at all, from which the minimum adult length may be assumed to be 20 mm.

The head end is somewhat pointed, with a poorly defined shoulder, the tail bluntly rounded, the lateral margins even or slightly wavy. In fresh specimens the color is a uniform deep pink—ham color—and to the touch and the unaided eye the integument appears smooth with transverse lines more or less prominent according to the state of muscular contraction.

The Cuticula.—Microscopically, this is seen to consist of a basement membrane overlaid with the true cuticula, which under high magnification shows fine striae vertical to the plane of the surface. It is somewhat thicker on the dorsal (19 to 34μ) than on the ventral surface (15-27), the maximum being at the level of the upper border of the acetabulum, from which point it becomes thinner in both directions. True cuticula similar to that covering the surface of the body, but thinner, extends for varying distances into all the organs which open externally.

The entire ventral surface, including the narrow strip anterior to the oral sucker, is armed with spines, which are most numerous in the acetabular region (Pl. XVII, *A*). These spines are deeply imbedded in the cuticula, their bases resting on or in the basement membrane,

and are directed caudad at an angle of 30-45 degrees, the merest tip projecting beyond the surface. They are arranged, somewhat irregularly, in alternating transverse rows (Pl. XIII, *B*) which in the most thickly covered regions may be almost in contact, but will average 10 to 15μ apart, and toward the tail may be separated by 160μ or more. Similarly, in the transverse direction the spines are practically in contact over the acetabulum, but toward the tail the interval between the bases of adjoining spines varies from 19 to 40μ.

The Spines.—The spine itself (Pl. XIII) is a scale-like structure, 25 to 30μ wide at the base, and 30 to 34μ long. The sides converge a trifle toward the tip which is bluntly rounded and curved backward. The upper surface of the spine is flat or, especially toward the tip, slightly concave. In the lower surface the curvature is somewhat greater, giving to a transverse section a crescentic outline. The body of the scale increases in thickness from a thin edge at the tip to 10 to 13μ at the base where it flares out rapidly like the thorn of a rose. The surface of the base, where it is attached to the basement membrane, presents two, three or rarely four cusps, causing a transverse section through it to appear like a group of rounded or irregular bodies. The size of the scales varies with the stage of development of the parasite, and with the location, being smallest around the oral sucker and the genital pore. Extremes in length were 22.8 and 41.8μ, other dimensions being in proportion.

The Musculature.—Muscular fibers occur in the walls of the various viscera, and are particularly well developed in the cirrus and the metraterm. In addition, the muscular system comprises the following:

1. *The dermo-muscular tube* lies immediately beneath the basement membrance of the cuticula, and consists of annular, oblique and longitudinal fibers. At rather frequent intervals are found other longitudinal and also dorso-ventral fibers (the parenchymal muscles), and certain fibers attaching various viscera, especially the two suckers and the shell gland to the dermo-muscular tube.

2. *The oral sucker* is situated at the anterior extremity, its orifice being sub-terminal on the ventral surface, and its long axis, which is continuous with that of the pre-pharynx and pharynx, being oblique to the surface (Pl. XII, *B*). In younger specimens the sucker is nearly globular, but with age the transverse diameter becomes longer, and the dorso-ventral shorter relative to the longitudinal. In well developed specimens these diameters will range from about 0.5 to 0.7 mm. The oral orifice is normally circular, 0.3-0.4 mm. in diameter. The entire organ (and this applies as well to the pharynx and the ventral sucker) is enclosed in a capsule and suspended by means of several processes

of muscular and connective tissue in a sinus of the excretory system, doubtless in order to facilitate muscular contractions. In addition to this, considerable motion in its longitudinal axis, whereby the organ may be partially extruded through the oral ring, is made possible by its free attachment to the ring by means of an eversible collar of cuticula and connective tissue, and by the interposition of a collapsible portion of the alimentary tract—the pre-pharynx—between the oral sucker and the pharynx. Whether this freedom of motion is used for locomotion as well as for feeding is not determined, but seems probable.

2. *The pre-pharynx,* whose function seems to be to permit motion of the oral sucker, is surrounded by a well developed muscle consisting of circular fibers, and extending from the upper surface of the pharynx to about the middle of the oral sucker, which it is thus able powerfully to reinforce (Pl. XII, *B*).

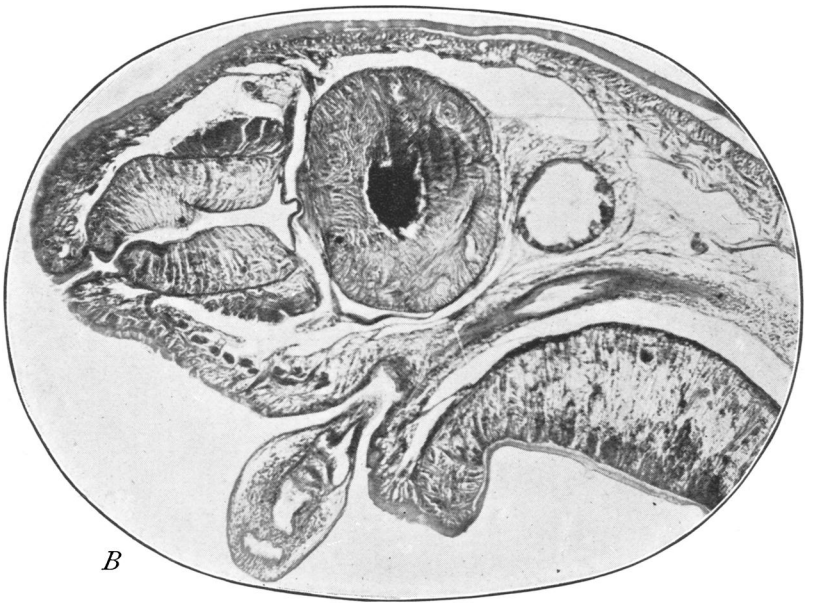
3. *The pharynx,* which surrounds the alimentary tract just prior to its bifurcation and lies beneath the dermo-muscular tube posteriorly, is a spheroidal mass of radial and circular fibers measuring from 0.40 to 0.75 mm. antero-posteriorly, and from 0.70 to 0.99 mm. transversely.

4. *The ventral sucker* is a powerfully developed, bell-shaped organ, situated near the anterior extremity (1.2 to 1.8 mm. from the tip) and so placed that its long diameter and the plane of its orifice are oblique to the ventral surface, the anterior lip of the orifice being somewhat longer than the posterior. Its total length varies from 2.3 to 3.2 mm., and its greatest diameter from 1.7 to 2.5 mm., the dorso-ventral diameter being somewhat shorter than the transverse. The orifice, normally circular with a diameter of 1.0 to 1.5 mm., is in preserved specimens often transversely ellipitical or irregularly heart-shaped. The acetabular cavity is lined throughout with an extension of the cuticula.

The alimentary tract extends as a single tube from the oral aperture to just beyond the pharynx where it bifurcates, forming the two ceca which proceed laterally to the outer margins of the acetabulum, at which points they bend sharply caudad and follow a sinuous course to the posterior extremity, ending blindly near each other. This course is marked by two main inward curves, viz., at the level of the shell gland, and between the testes; but the ceca follow the outline of the viscera they enclose and hence the number and degree of their curves depend in part upon the development of the individual, and the state of its muscular contraction. The lumen of this tract varies considerably in size. At the oral aperture it appears in sections as a transverse slit, 0.2 to 0.3 mm. by 0.085 to 0.100 mm., but immediately expands, then narrows, to expand again within the pharynx, from

e *f* *g* *i* *j* *k*

A



B

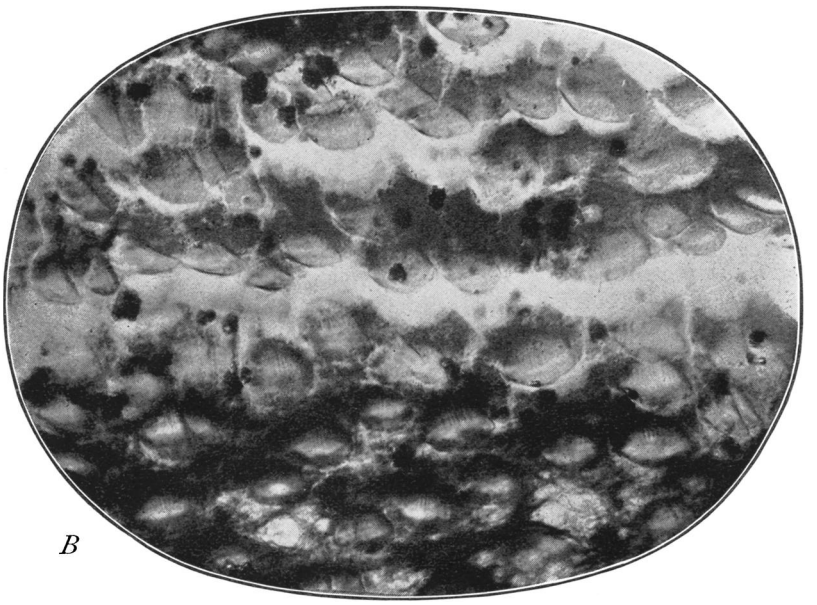
PLATE XII

EXPLANATION OF PLATE

A. Varieties of the fluke encountered in Shaohing.

B. Anterior extremity, showing especially the sinuses surrounding oral sucker, pharynx, and ventral sucker; nerve cell in pharynx; cuticula stripping off and also lining of oral and ventral suckers. Sagittal section. (× 68.)

A



B

PLATE XIII

EXPLANATION OF PLATE

A. Spines embedded in cuticula, and being stripped off with it. Sagittal section. (×450.)

B. Ventral spines, arranged in alternate rows. Section in ventral plane. (×450.)

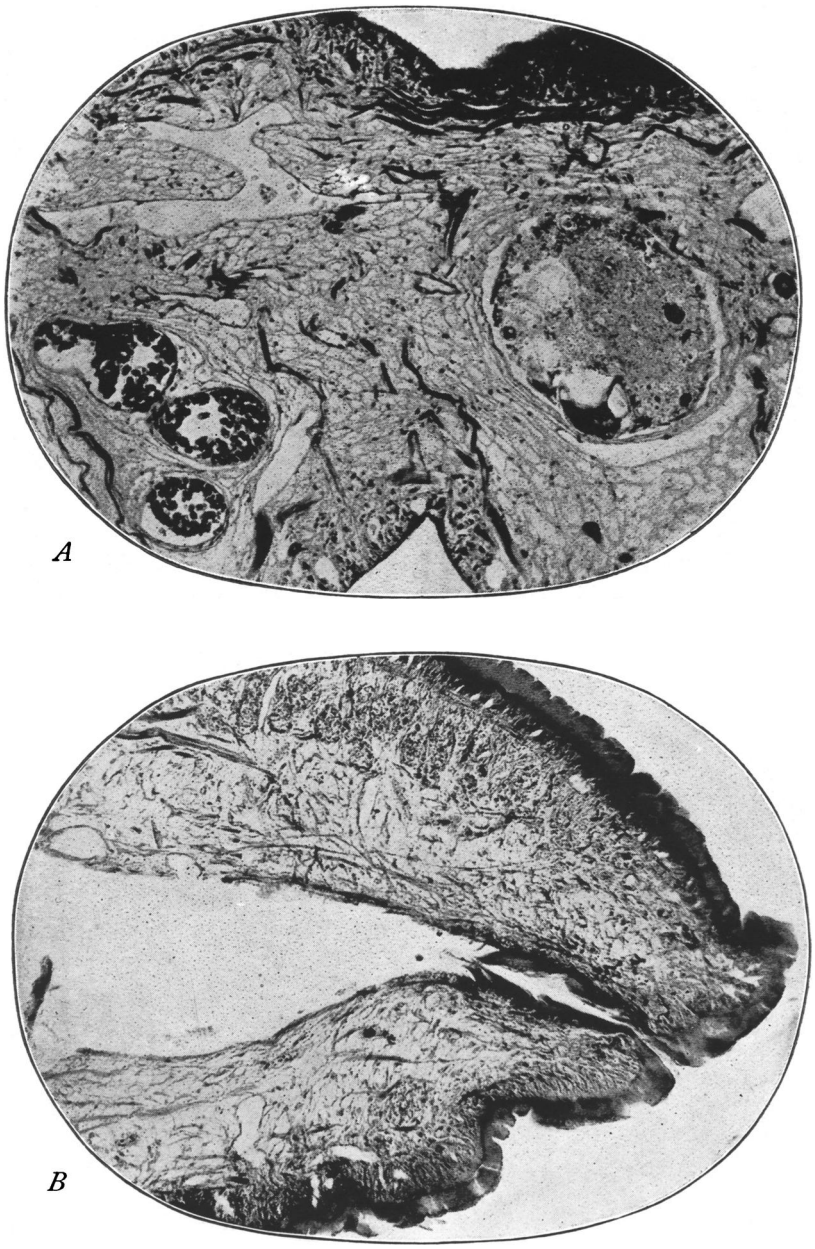


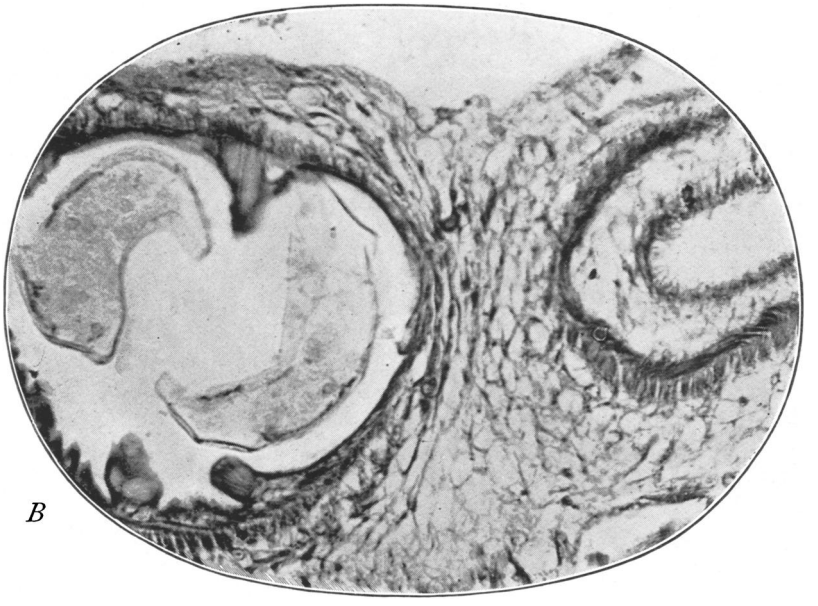
PLATE XIV

EXPLANATION OF PLATE

A. From fluke F showing entire absence of cuticula. Also coils of ovary at left, nerve cell at right. Sagittal section. (× 68.)

B. Posterior extremity, showing excretory vesicle and duct. Sagittal section. (× 68.)

A



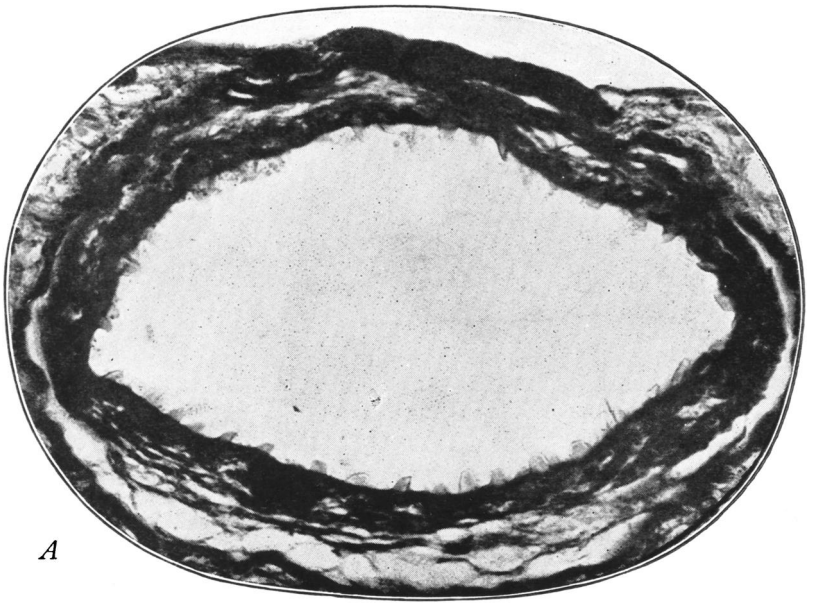
B

PLATE XV

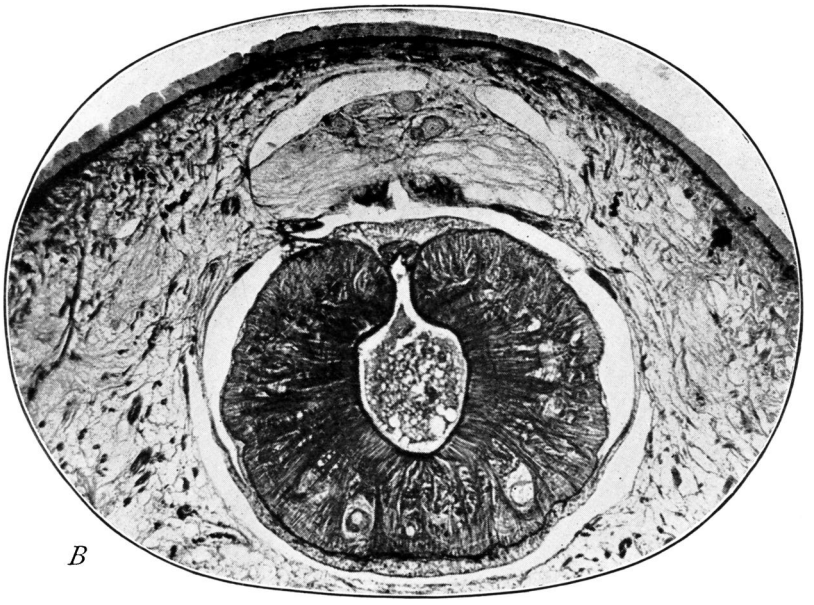
EXPLANATION OF PLATE

A. Shell gland, with vas efferens at left, coils of Laurer's canal above, junction of common yolk duct with oötype in center, and vitelline receptacle below. Ventral section. (× 90.)

B. Metraterm and everted cirrus just below level of genital pore, showing 3 large spines in metraterm, also spines of cirrus and pre-cirral canal. Ventral section. (× 360.)



A



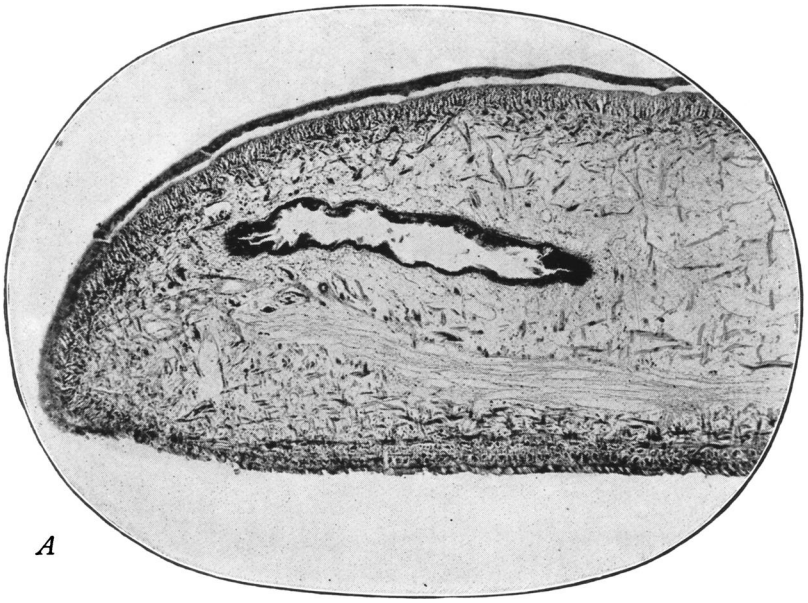
B

PLATE XVI

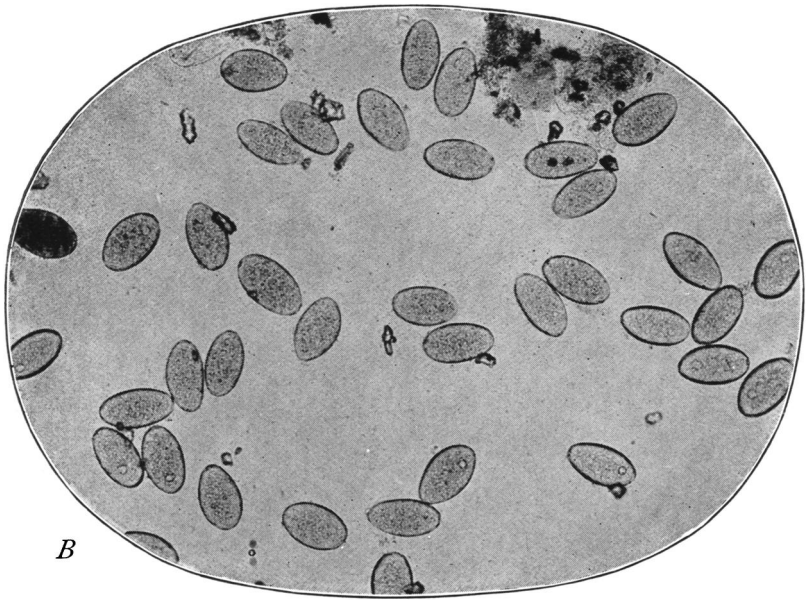
EXPLANATION OF PLATE

A. Pre-cirral canal, showing spines. (× 450.)

B. Three nerve cells at base of oral suckers and others in pharynx. Note how freely pharynx is suspended in sinus of excretory system. (× 90.)



A



B

PLATE XVII

EXPLANATION OF PLATE

A. Sagittal section, showing nerve trunk at level of ventral sucker with a nerve cell near anterior extremity. Cecum at right. Note cuticula with spines on ventral surface, stripping off on dorsum. (×45.)

B. Group of eggs from feces. (×68.)

which it emerges as a tube 0.135 to 0.175 mm. in diameter. This portion of the tract, the esophagus proper, is very short (85 to 100μ), as the transverse portion of the ceca lies practically in juxtaposition with the pharynx. The diameter of the ceca is not constant, but at the acetabulum approximates 230μ and diminishes somewhat toward the tail. Up to the point of its bifurcation this canal is lined with an extension of the cuticula; beyond that point wtih tall columnar epithelium arranged in several (5-9) longitudinal ridges, which give to it a characteristic stellate appearance on cross section.

The excretory system begins immediately under the basement membrane of the cuticula as small spaces or canals which coalesce to form larger ones, and eventually empty into the excretory vesicle. In the anterior part of the body, three main trunks — a central and two lateral—gather up the smaller branches and unite at the lower border of the shell gland to form the excretory vesicle, which occupies the central portion of the fluke from this point to the extremity, with a diameter about one third of the thickness of the body at that part — approximately 500 or 600μ. Throughout its extent it receives transverse and oblique tributaries, and from its caudal extremity a short, straight duct, 10 or 12μ in diameter, leads to the external orifice on the posterior surface, about 300μ from the tip (Pl. XIV, *B*). This excretory pore is provided with a sphincter in the dermo-muscular tube; the duct is lined throughout with cuticula, the vesicle with tall columnar epithelium, and the other branches of the system with a single layer of flattened cells.

Striking features of the anatomy of the fluke are the large sinuses surrounding the oral sucker, the ventral sucker, the pharynx, and to a less extent the shell gland and other organs, and communicating directly and freely with the main trunks of the excretory system. It seems reasonable to suppose that in addition to their excretory function these sinuses are analogous to the synovial or pleural sacs of mammals (Pl. XII, *B*, and Pl. XVI, *B*).

The Male Reproductive System.—The testes, two in number, consist of thick tubes, 0.6 or 0.7 mm. in diameter, and about one third the width of the fluke in length, which lie one behind the other ventral to the excretory vesicle, and with their branches occupy the greater part of the posterior half of the body. The branching is frequent and usually dichotomous, the finest divisions ranging from 116 to 200μ or more in diameter. From each main trunk a vas deferens arises, and these pass forward over the surface of the shell gland on either side (Pl. XV, *B*), and converge to pierce the cirrus sac and empty into the posterior tip of the primary seminal vesicle. This point, the beginning of the cirrus sac, is usually easily made out in cleared specimens, and

lies ordinarily about midway between the shell gland and the tip of the acetabulum, but may be very much nearer the former.

The cirrus sac consists of muscular and fibrous tissue, and contains from behind forward the following structures: seminal vesicles, ejaculatory duct, cirrus and pre-cirral canal, the last three of these constituting the vas deferens. The first portion of the sac terminating at the tip of the acetabulum is more or less convoluted, and has a diameter of 500-700μ. The second portion containing the vas deferens follows a straight course longitudinally, lying in apposition with the acetabulum in front, and with the metraterm to the left. Anteriorly, its walls blend with those of the genital atrium.

The seminal vesicles are two more or less convoluted tubes, lying side by side within the first portion of the cirrus sac. One of these vesicles, here termed the primary vesicle, extends caudad slightly farther than the other and receives the vasa efferentia. Its distal extremity empties into the lumen of the secondary vesicle (the so-called cecal appendage of the former descriptions) where it narrows to form the ejaculatory duct, but is directed caudad. Thus both vesicles are practically always full of sperm. Both vesicles are lined with a cylindrical epithelium.

The spermatozoön possesses the usual form—namely, a spindle-shaped body and a filamentous tail, with a total length of 6 or 7μ. The ejaculatory duct, approximately the first half of the vas, is continuous with the seminal vesicle behind and the cirrus in front, and differs from the former only in being straight and smaller in diameter (140-200μ). The cirrus forms about half of the remaining portion of the vas. The muscular fibers in its walls are notably developed and it is lined with very small and delicate spines, which hardly take the stain for chitin (Pl. XV, *B*). The distal extremity of the cirrus protrudes into the pre-cirral canal very much as the human cervix uteri extends into the vagina.

The pre-cirral canal which opens anteriorly into the base of the genital atrium, is closely set throughout with spines similar to the cuticular variety, but much smaller and more delicate, being 6.5x7μ (Pl. XVI, *A*). When the cirrus is protruded this canal is entirely evaginated, forming then an outer coat enclosing the whole length of that organ except its tip. This doubtless will account for the statement by Stephens that the cirrus "is beset with very fine spines except at either extremity"; for the spines in the pre-cirral canal do not extend over the portion of the cirrus which projects into it (Pl. XV, *B*).

The genital atrium, into the bottom of which the pre-cirral canal and the metraterm empty side by side, is 250 to 300μ deep, and when relaxed appears as a transverse slit some 400 to 500μ wide. During

extrusion of the cirrus, however, that organ completely fills the lumen, and considerably distends it, giving it a cylindrical form. The atrium is lined with cuticula, but without spines.

The Female Reproductive System.—The vitelline glands are symmetrical structures, occupying the lateral portions of the fluke, and extend from the level of the acetabulum to the posterior extremity where they meet in the middle line. They lie superficially, immediately within the dermo-muscular tube, extending inward on both the ventral and dorsal surfaces for about one sixth of the width of the fluke, and thus enwrap to a certain extent the outlying parts of the testes, uterus, etc. Their acini are round or oval, and lined with large celled epithelium (18 to 20μ), with prominent round nuclei (4 to 5μ) and numerous refractile granules (2 to 2.5μ). On each side the yolk substance is collected by numerous tubules into an anterior and posterior longitudinal tubule, which unite to form the transverse yolk duct, 100μ in diameter. When near the shell gland the transverse ducts bend dorsalward to enter it in its dorsal and posterior portion, and expand at their junction within it to form the vitelline receptable (Pl. XV, *A*), from which the common yolk duct, a small tubule, extends upwards and unites with the short oviduct to form the oötype.

The shell gland is an elastic body surrounded by a capsule of connective tissue, into which the parenchymal muscles are more or less blended. It is generally ovoid in shape with the long axis oblique to the midline of the body, the anterior end to which the ovary is attached being to the right of this line. In the smaller flukes it is situated but a millimeter or less anterior to the mid point of the body, but in the longest specimens lies at about the junction of the anterior and middle thirds. The cells of this gland are generally spindle-shaped (10x22μ), but some are rounded (10 to 15μ in diameter) with large, round, deeply staining nuclei (5μ in diameter). These are gathered in a peripheral zone 100μ or more in thickness, from which delicate processes converge toward the oötype.

The ovary consists of three stems—superior, middle and inferior — the outer ends of which are closely branched, while inwardly they soon merge into a common mass lying upon the upper right hand portion of the shell gland near the ventral surface. From this mass a small duct, the oviduct, sinks into the shell gland, takes a convoluted course to the posterior part of the gland where it turns toward the opposite side, gives off Laurer's canal, and immediately afterwards is joined by the common yolk duct to form the oötype. The cells of the ovary, or ova proper, stain a dark reddish brown with van Gieson, are generally nearly round (12 to 15μ in diameter), with large, round, deeply staining nucleus (7 to 8μ) and nucleolus (5μ).

The oötype is a straight cylindrical tube, 100 to 135μ in diameter, extending transversely through the substance of the shell gland, and is continuous at either end with the oviduct and the uterus, respectively. It is lined with a layer of tall columnar cells lying upon a basement membrane, and in stained sections is surrounded to a depth of 50 to 60μ with deeply stained, radially arranged lines—the terminal portions of the processes of the cells of the shell gland — which give to it a characteristic caterpillar-like appearance.

Laurer's canal is a small duct given off from the oviduct just before that canal unites with the common yolk duct to form the oötype. It follows a convoluted course, especially in that part within and adjacent to the shell gland, and empties on the posterior surface in the mid line at a point a millimeter or so in a straight line from its origin. It is lined throughout with an extension of the cuticula, and is provided with a sphincter at its distal extremity. In general, its lumen is about 12μ, but frequent enlargements or sacculations of the tube occur, having twice or three times that diameter. There is no trace of any receptaculum seminis. Sperm was not found in any of the cases examined, but only a few cells from the ovary, and still more rarely yolk cells. Probably these were abnormally forced into the canal during the death spasm.

The uterus in the first part of its course is differentiated from the oötype by the absence of the caterpillar-like rays, and by its convoluted course. It leaves the shell gland near the upper left hand portion of its ventral surface, and arranged in loose coils occupies most of the anterior portion of the fluke as far as the acetabulum. In the larger flukes it is packed with eggs, and may attain a diameter of 500μ or more. The first portion of the uterus is lined with cylindrical epithelium — as is also the oviduct — but in the central portion this is reduced to a layer of thin, flattened cells with delicate processes projecting into the lumen like spines in appearance except that they do not stain. At the tip of the acetabulum the uterus merges into the metraterm, becoming reduced in size (about 150μ), the walls are thickened, muscular fibers especially being increased and the lining becomes identical with the outer cuticula, and is armed with stout spines measuring approximately 23x28μ (Pl. XV, *B*). These have been found embedded in the cuticule from the outer termination of the metraterm for somewhat over two thirds of its length and may possibly normally be found throughout its entire extent. Throughout its course the metraterm lies upon the acetabulum in juxtaposition with the vas and to its left.

The ovum is an ellipsoid, rather bluntly rounded at either pole, and measuring normally 130 to 140μ by 80 to 85μ. The shell is clear and

thin, the operculum so delicate as to be made out only with difficulty in many instances, and the contents consist of a large number of yolk cells with usually but a single germinal cell situated towards the operculum from the center. Considerable variations in size and shape are met with, though they are not common in eggs deposited in the normal manner, as is apparent from a study of Table 2, Graphs 4, 5, 6, and Pl. XVII, *B;* from these it also appears that the normal egg as found in feces is not so pointed toward the poles as it has been figured hitherto.

The Nervous System —The nervous system may be divided into a peripheral and a visceral portion. The peripheral portion consists of a group of numerous ganglion cells clustered around the lower portion of the oral sucker (Pl. XVI, *B*), from which three nerves proceed on each side.

1. The ventral nerves pass rapidly forward toward the surface, and from the level of the acetabular orifice to their termination at the posterior extremity are found immediately below the dermo-muscular tube and slightly within the general course of the ceca (Pl. XVII, *A*). Anterior to the acetabulum they are united by three commissures, one at the base of the pharynx, and the others respectively above and below the transverse portion of the ceca. Throughout the remainder of their extent at intervals of from 300 to 700μ they give off median and lateral branches which, with corresponding branches from the other nerves, are distributed to the entire periphery. Opposite the acetabulum the ventral nerves are 135μ or more wide and somewhat less in thickness, and taper gradually toward the tail.

2. The dorsal nerves are superficial throughout their entire course, are united by a single commissure behind the cecum, and are somewhat smaller than the ventral nerves, but similar in their branchings and distribution.

3. The lateral nerves arise from the common origin and by a plexus of three or four branches from the ventral nerves on either side, and are distributed to the shoulders, terminating at about the level of the acetabulum.

Small bundles of fibers from the ventral nerves have been traced to the acetabulum and pharynx, and doubtless the two portions of the nervous system are intimately correlated, though this has not been demonstrated. But in general it is evident the portion of the system just described is concerned primarily with the innervation of the periphery, including the dermo-muscular tube.

4. The cells referred to as "ganglion cells" above, are elliptical cells about 38μ long, and 30μ in their short diameter. The nucleus is large and round, and the cytoplasm finely reticulated. In some

instances fibers have been seen to pass from the cell into the nerve bundle. As stated above, a large group of these cells is to be found around the base of the oral sucker, but single cells occur also at various points along the course of the nerves.

The visceral portion of the nervous system consists of round or oval cells similar to the ganglion cells of the peripheral portion, but without visible fibers leading from them. They occur in great numbers throughout the body, being most numerous in the powerfully developed muscles (oral sucker, pharynx, and ventral sucker), and in the cirrus and the metraterm; and to a less extent about the other viscera.

The foregoing detailed description may be summarized as follows: *Fasciolopsis buski* is a flat, elongated fluke, presenting great variation in size, but averaging about 30x12x2 mm. It is deep pink in color, and surface appears smooth except under microscope when ventral surface is seen to be beset thickly with spines. Oral sucker subterminal on ventral surface; ventral sucker powerful and near anterior extremity. Genital pore just anterior to ventral sucker. Vitellaria are racemose glands occupying lateral portions from ventral sucker to tail. Shell gland oval in mid line of body somewhat anterior to mid point. Uterus in loose coils occupies anterior portion of fluke, its terminal portion, the metraterm, being spined and emptying into the common genital atrium beside the pre-cirral canal. Ovary, closely branched, attached to shell gland on the right. Testes, two in number, closely branched, and lying one behind the other, occupy most of the body posterior to the shell gland. Cirrus and pre-cirral canal lie parallel with metraterm, and are armed with fine spines. Excretory vesicle large, with many transverse and oblique branches, and empties on doral surface, near posterior extremity.

CONCLUSIONS

1. Infestation with *Fasciolopsis buski* is to be regarded as a serious disease; and where local conditions favor it becomes of considerable importance from the standpoint of public health.

2. The most notable symptoms are general weakness, diarrhea, anemia, and edema. The rapid accumulation of fluid in the body may be accompanied by a pronounced suppression of urine without evidence of renal involvement.

3. Contrary to certain authorities, fever is not noted, except in complicated cases.

4. The parasite shows great variation in morphology, but withal such gradation in variation as to justify including the forms now described as *F. rathouisi* and *F. goddardi* in the species *F. buski*. On

account of the close similarity of *F. fülleborni,* it would appear desirable to subject this species also to further investigation.

5. Ventral cuticular spines are characteristic of *F. buski,* and probably of the entire genus.

6. Cirrus and metraterm are spined in *F. buski,* and therefore Raillet's description of the family Fasciolidae, if it is still to include Fasciolopsis, needs to be revised accordingly.

ACKNOWLEDGMENTS

Grateful acknowledgement should be made here of my indebtedness to the China Medical Board of the Rockefeller Foundation for the grant of a fellowship without which this investigation would have been impossible, and to Prof. E. E. Tyzzer of the Department of Comparative Pathology of Harvard University under whose direction it has been carried on, and whose cordial interest in the work has been manifested in many helpful ways. It is a pleasure to express my indebtedness also to my colleague, Dr. C. H. Barlow, of Shaohing, China, for aid in securing some of the material; to Prof. H. B. Ward of the University of Illinois for helpful suggestions, and to Prof. F. B. Mallory of Harvard University, and his able assistant, Miss Lillian M. Leavitt for the fine series of microphotographs which accompany this article.